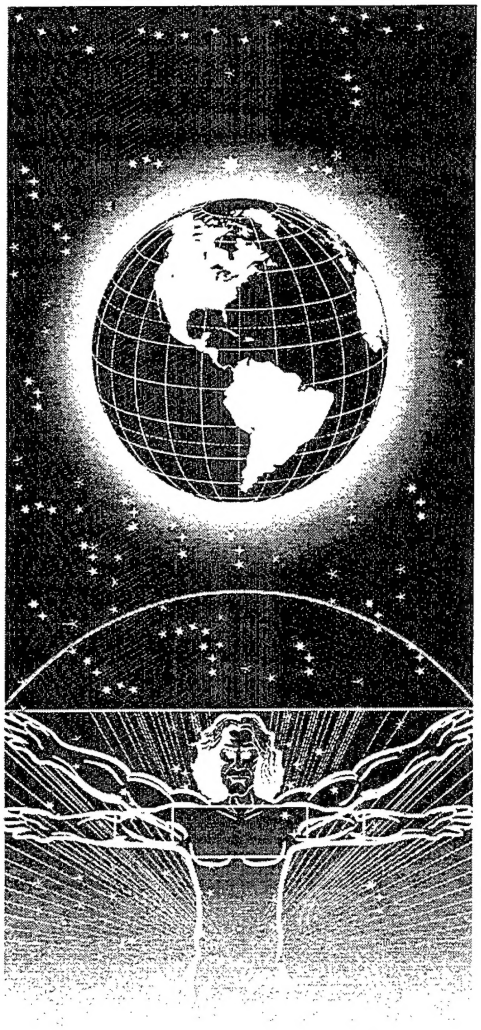
AL/OE-TR-1995-0090



UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

Development of Microdialysis Probe Method for Partition Coefficient Determination for Pharmacokinetic Modeling

Melanie C. Caracci

GEO-CENTERS, Inc.
7 Wells Avenue
Newton Centre MA 02159

R. S. Geary
C. M. Wall

Southwest Research Institute
6620 Culebra Road
San Antonio TX 78238

Gary W. Jepson

Toxicology Division, Occupational and
Environmental Health Directorate, Armstrong Laboratory
Wright-Patterson AFB OH 45433-7400

May 1995

Approved for public release;

distribution is unlimited.

Occupational and Environmental Health Directorate
Toxicology Division
2856 G Street
Wright-Patterson AFB OH 45433-7400

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Armstrong Laboratory. Additional copies may be purchased from:

NATIONAL TECHNICAL INFORMATION SERVICE
5285 PORT ROYAL ROAD
SPRINGFIELD, VIRGINIA 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

DEFENSE TECHNICAL INFORMATION CENTER
8725 JOHN J. KINGMAN RD STE 0944
FT BELVOIR VA 22060-6218

DISCLAIMER

This Technical Report is published as received and has not been edited by the Technical Editing Staff of the Armstrong Laboratory.

TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1997-0090

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 1995	Interim - May 1994 - May 1995

4. TITLE AND SUBTITLE
Development of Microdialysis Probe Method for Partition Coefficient Determination for Pharmacokinetic Modeling

5. FUNDING NUMBERS
Contract DAMD17-93-C-3006
PE 63716D
PR 4223
TA 4223OT
WU 7757A102

6. AUTHOR(S)
M. C. Caracci, R. S. Geary, C. M. Wall and G. W. Jepson

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
GEO-CENTERS, Inc.
7 Wells Avenue
Newton Centre, MA 02159

Southwest Research Institute
6620 Culebra Road
San Antonio, TX 78238

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Armstrong Laboratory, Occupational and Environmental Health Directorate
Toxicology Division, Human Systems Center
Air Force Materiel Command
Wright-Patterson AFB OH 45433-7400

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
AL/OE-TR-1995-0090

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The value of any Physiologically Based Pharmacokinetic (PBPK) model depends largely on the quality of the parameter estimates entered into the model. One of the critically important parameters requiring estimation is the partition coefficient. Commonly in the field of pharmacokinetics, partition coefficients are determined by the modified version of the vial equilibration technique. This method is an *in vitro* method in which an animal must be sacrificed, and the tissue and blood harvested to complete the procedure. In the vial equilibration technique, certain physiological aspects must be compromised as the tissues are isolated from a living system. However, a method to determine various partition coefficients *in vivo* would reduce or eliminate the compromises inherent in the *in vitro* approach. This technical report describes a method that was developed to determine *in vivo* partition coefficients by using microdialysis probes. If the microdialysis probe method of determining partition coefficients *in vivo* is shown to be valid, then it could be used in collaboration with the vial equilibration method to provide partition coefficients of better quality than is currently available. Therefore, the data produced by the pharmacokinetic models in which partition coefficients are used would be greatly enhanced.

14. SUBJECT TERMS
Partition coefficients
Microdialysis probe
Physiologically based pharmacokinetic (PBPK) model

15. NUMBER OF PAGES
22

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

THIS PAGE INTENTIONALLY LEFT BLANK

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

The value of any Physiologically Based Pharmacokinetic (PBPK) model depends largely on the quality of the parameter estimates entered into the model. One of the critically important parameters requiring estimation is the partition coefficient. Commonly in the field of pharmacokinetics, partition coefficients are determined by the modified version of the vial equilibration technique [1,2]. This method is an *in vitro* method in which an animal must be sacrificed, and the tissue and blood harvested to complete the procedure. In the vial equilibration technique, certain physiological aspects must be compromised as the tissues are isolated from a living system. Furthermore chemical is often lost in tissue handling and analysis. However, a method to determine various partition coefficients *in vivo* would reduce or eliminate the compromises inherent in the *in vitro* approach.

The method proposed in this technical report utilizes microdialysis probes to determine tissue/blood *in vivo* partition coefficients in rats. The use of microdialysis probes is well established in brain studies [3,4,5] for determining neurochemical levels. Microdialysis probes consist of two "tubes" through which a perfusate is continuously pumped at a low flow rate. The tubes are linked by a dialysis membrane through which chemicals are driven by a concentration gradient from the tissue into the perfusate. As a result, the chemical concentration of extracellular space of a living animal could be sampled without disturbing the fluid balance [6], see figure 1.

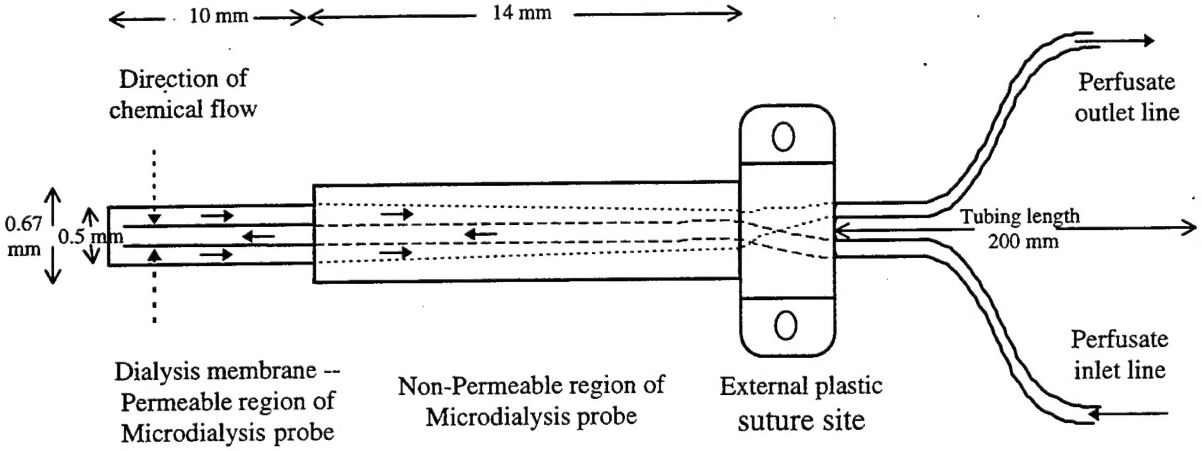


Figure 1: Diagram of Microdialysis probe.

If the microdialysis probe method of determining partition coefficients *in vivo* is shown to be valid, then it could be used in collaboration with the vial equilibration method to provide partition coefficients of better quality than is currently available. Therefore, the data produced by the pharmacokinetic models in which partition coefficients are used would be greatly enhanced.

METHODS AND MATERIALS

Materials

The model chemical in this study was Trichloroethylene (TCE); it was of 99.5% purity and was purchased from Aldrich. Also used in this study were the following chemicals: 1,1,2-Trichloroethane (Technical grade) purchased from FisherChemical, *tert*-Butyl methyl ether (99.8% purity) purchased from Aldrich, and Lactated Ringer's solution which was purchased from McGaw Inc.

Method for microdialysis probes preparation

Prior to use, the microdialysis probe is connected to a 2.5 ml glass syringe of Lactated Ringers with a 23 gauge needle hub. The Lactated Ringers is pumped through the microdialysis probe for 30 minutes at a rate of 5.0 µl/min by a microinjection pump. This procedure is completed to remove the glycerin that the microdialysis probes are stored in during delivery.

Method for in vitro determination of standard curve

To determine a microdialysis probe/Trichloroethylene (TCE) standard curve, the microdialysis probes were individually suspended in concentrations of TCE/Lactated Ringers ranging from 0 - 60 µg/ml, and samples were collected via the microdialysis probe. As figure 2 shows, the microdialysis probes were exposed to the solutions in 20 ml scintillation vials which were maintained at 37°C by the use of a water bath. The scintillation vials were capped with screw caps together with 20 mm Teflon/Rubber septas through which the microdialysis probes were inserted. Two Intramedic Non-radiopaque polyethylene tubes were inserted through the septas to completely fill the vials with the Trichloroethylene/Lactated Ringers solution. The microinjection pump was then started and Lactated Ringers was pumped through the microdialysis probes at a rate of 5.0 µl/min. Samples from the microdialysis probes were collected in microcentrifuge tubes containing 100 µl of 50 µg/ml of 1,1,2-Trichloroethane/*tert*-Butyl methyl ether until steady state was obtained. The microcentrifuge tubes were kept on ice until analysis. The samples from the microdialysis probes were analyzed by the method described below in the section entitled, "Sample Analysis". At each concentration completed, the average of the steady-state area counts was calculated. A standard curve depicting the resulting average area counts versus concentration was compiled.

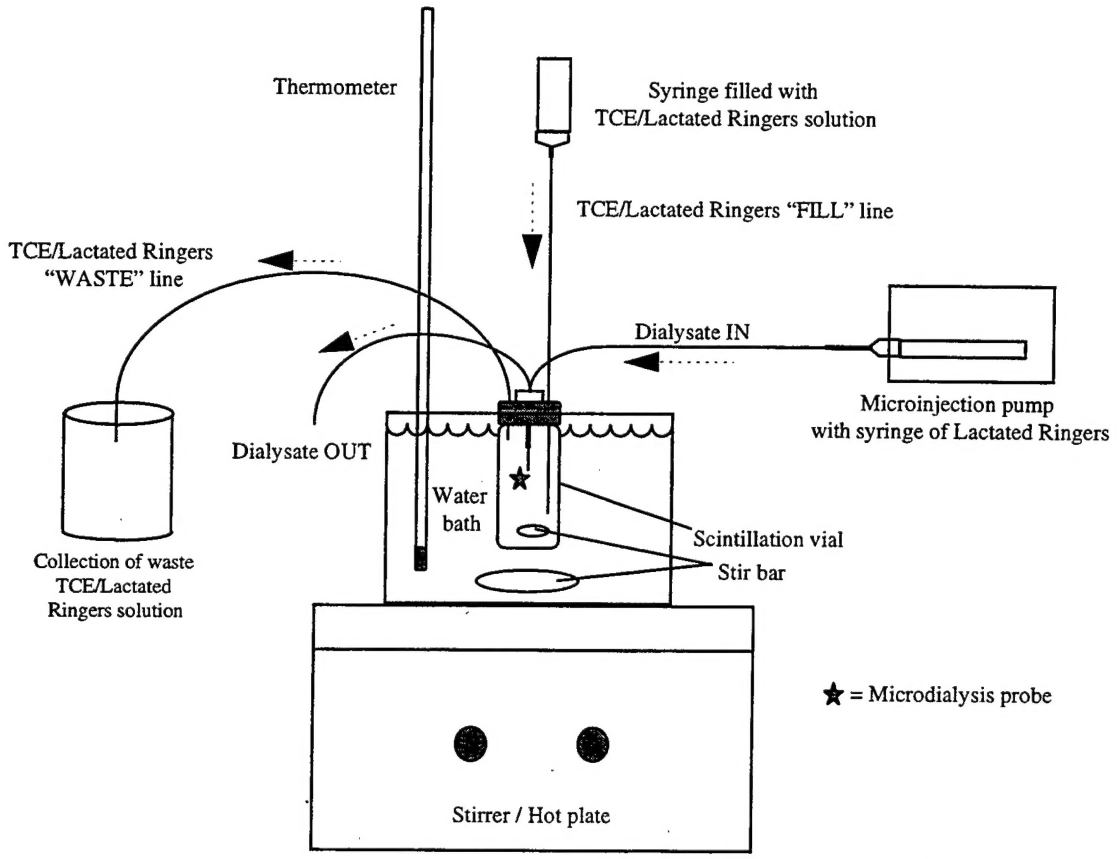


Figure 2: Schematic of *in vitro* apparatus used to determine microdialysis probe standard curve for trichloroethylene.

Method for in vitro validation and relative recovery estimation

Validation of the theory that the microdialysis probe could collect trichloroethylene from a tissue was first tested *in vitro*. A kidney was removed from an unexposed F-344 male rat which had been previously asphyxiated with Carbon Dioxide. The excised kidney was cored by using a 5 mm in diameter hole punch. The microdialysis probe was then inserted lengthwise through the center of the kidney core and tied into place with 4-0 suture silk. The microdialysis probe and kidney were suspended in TCE/Lactated Ringers solution similar to the method described to determine the standard curve. Fifty microliter samples from the microdialysis probes were collected at a flow rate of 5.0 µl/min in microcentrifuge tubes containing 100 µl of 50 µg/ml of 1,1,2-Trichloroethane/*tert*-Butyl methyl ether until steady state was reached. The microcentrifuge tubes were kept on ice until analysis. The samples from the microdialysis probes were analyzed by the method described below in the section entitled, "Sample Analysis".

Relative recovery shows the relationship between the concentration of analyte in the dialysate (TCE in the probe samples) and the concentration of analyte in the original sample (TCE in the exposure solutions). Relative recovery is inversely proportional to the microdialysis probe flow rate, such that as the flow rate approaches zero, the relative recovery will approach 100% [7]. It is expressed as a percentage and is used as a

correction factor for the samples collected *in vivo*. In order to determine the relative recovery, a microdialysis probe was suspended in 20 µg/ml and 40 µg/ml of TCE/Lactated Ringers, similar to the method used to determine the standard curve. Probe dialysate was collected at a flow rate of 5.0 µl/min into microcentrifuge tubes containing 100 µl of 50 µg/ml of 1,1,2-Trichloroethane/*tert*-Butyl methyl ether until steady state was reached. In addition, 50 µl of the exposure solution was sampled through the polyethylene "waste" line, see figure 2, and placed in microcentrifuge tubes containing 100 µl of 50 µg/ml of 1,1,2-Trichloroethane/*tert*-Butyl. The microcentrifuge tubes were kept on ice until analysis. All the samples were analyzed together as described below in the section entitled, "Sample Analysis".

Method for in vivo partition coefficients determination

Throughout the *in vivo* procedure, two gas chromatographs (GC) were utilized. One GC was used solely to monitor the exposure concentration of trichloroethylene (TCE) during the nose-only exposure. Another GC was utilized to analyze the samples produced by the microdialysis probes following extraction.

Exposure Set-up

Figure 3 is a schematic of the nose-only exposure system used in this procedure. The system was designed such that an anesthetized rat could be exposed to a gaseous chemical while still allowing for maximum accessibility to the rat by the operator. Except for the air tank and the GC, the entire exposure system was placed into a ventilation hood. The apparatus was set up so that the dynamic flow of the hood was from the tail of the rat to the nose, and then away from the body to eliminate the possibility of vapor TCE entering the microdialysis probes directly.

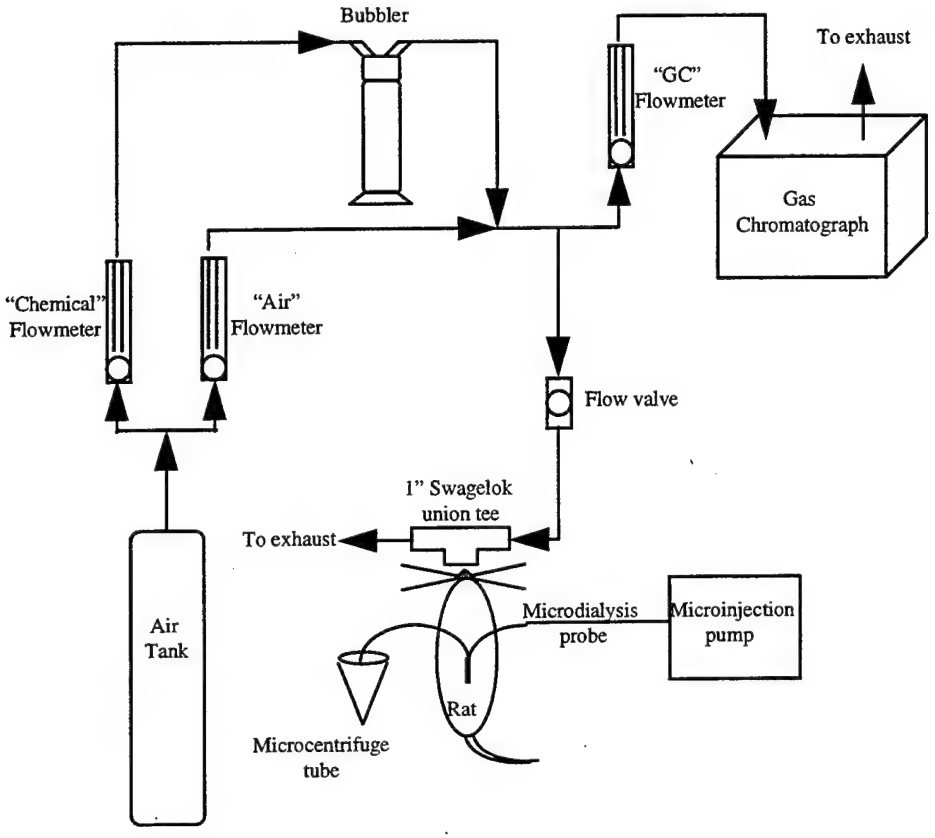


Figure 3: Schematic of nose-only exposure system utilized during *in vivo* partition coefficient determination.

Approximately 25 - 50 ml of TCE was placed into the bubbler and heated to 50°C. The flow rates to the bubbler and fresh air are individually controlled prior to mixing, by the 'chemical' flowmeter and the 'air' flowmeter respectively, which allows for operator control of the exposure concentration.

Throughout the procedure, the exposure concentration was analyzed by gas chromatography three consecutive times every ten minutes. A Hewlett Packard model 5880 gas chromatograph with an OV-101 column was used to analyze gaseous TCE via a flame ionization detector (FID). GC conditions were as follows:

Oven Temp:	120 °C
Injector Temp:	175 °C
Detector Temp:	250 °C
Aux Temp:	150 °C
Attn:	4
Area Threshold:	0
TCE Retention Time:	1.19 minutes

As it took 20 - 30 minutes for the exposure concentration to get to the desired level, the system was started prior to animal exposure and run throughout the procedure.

Microdialysis probe implantation

One Fisher-344 male rat weighing between 200 and 275 grams was anesthetized with 60 mg/kg of Sodium Pentobarbital via intraperitoneal injection. After the anesthetic has taken effect, an incision was made into the neck and the fat cleared to expose the external jugular vein. Once the jugular vein was exposed, a tissue forceps was placed under the vein for control of the vein while implanting the probe. A 23 gauge needle was inserted into the vein, maintaining a level needle. Using the needle as a guide, the needle was instantaneously removed and the probe inserted into the vein [9]. The microdialysis probe was sutured into place by utilizing the plastic suture guide, see figure 1. The incision was closed using wound clips, externalizing the input and output lines of the microdialysis probe at the incision site. Another incision was made in the abdomen and one probe is inserted into each the kidney, liver and/or perirenal fat in a similar fashion. This incision is closed as above, allowing the input and output lines of the microdialysis probe to remain accessible. As the microinjection pump has a limit of three probes, only two of these tissues may be monitored in addition to the blood during a single exposure.

Exposure

For the exposure, the nose of the rat was placed into the one inch Union Tee of the exposure set-up through which the desired TCE concentration was flowing. Fifty microliter samples were collected from the output line of the probe at a rate of 5.0 µl/min in a microcentrifuge tube containing 100µl of 50µg/ml of 1,1,2-Trichloroethane/*tert*-Butyl methyl ether. The microcentrifuge tubes were kept on ice until analysis. Throughout the procedure, the exposure concentration was closely monitored and microdialysis probe samples collected every 10 minutes. As the rat was to remain anesthetized throughout the procedure, it was necessary to administer additional Sodium Pentobarbital, as needed. After the exposure was completed, the rat was euthanized by CO_2 asphyxiation.

Sample Analysis

Following the collection of microdialysis probe samples, the microcentrifuge tubes are vortexed, and then centrifuged at 11,000 rpm for 10 minutes. The ether layer was pipetted into a 200 µl target polyspring insert inside a 2 ml automatic injection vial, and sealed with a crimp top septum closure. These samples were analyzed on a Hewlett Packard model 5890 gas chromatograph equipped with a Vocol column and electron capture detector (ECD). The autosampler tray was equipped with a Coolant System and the samples were kept at 0 - 5 °C. GC conditions are as follows:

Oven Temp:	50 °C
Injector Temp:	175 °C
Detector Temp:	300 °C
Range:	3
Attn:	0
TCE Retention Time:	3.34 minutes
1,1,2 Trichloroethane Retention Time:	6.83 minutes

Partition Coefficient Calculation

Initially it was believed that the use of an internal standard was necessary to adjust for variations in the injection volume. Therefore, 1,1,2-Trichloroethane was used as the internal standard, and a solution of 50 µg/ml of 1,1,2-Trichloroethane/*tert*-Butyl methyl ether was used for sample collection instead of simply *tert*-Butyl methyl ether. The calculations to adjust for the variations in injection volume are listed below, however as time progressed it was noted that only minimal improvements were made as a result of the internal standard and likewise these calculations were discontinued.

1. Determine the average area counts for 1,1,2-Trichloroethane for all the samples collected.
2. For each sample, compute the ratio of the average 1,1,2-Trichloroethane area counts to the individual sample 1,1,2-Trichloroethane area counts. This should result in a number close to one.
3. For each sample, divide the trichloroethylene area counts by the ratio computed in step 2 to obtain the corrected trichloroethylene area counts.
4. Using a standard curve created previously, compute the concentration of trichloroethylene in each sample.

As the process of creating the 1,1,2-Trichloroethane/*tert*-Butyl methyl ether solution was relatively simple, it was continued throughout the experimental stages in case it was determined later that the process of adjustment was preferred. In order to calculate the *in vivo* tissue/blood partition coefficient, simply divide the average tissue concentration by the average blood concentration.

RESULTS

For each standard curve point, the average steady-state concentration was individually determined for each data set as described in the method section. Graphical results of the 20 µg/ml, 40 µg/ml and 60 µg/ml data points are shown in figures 4, 5, and 6, with the summary results shown in table 1. The resulting 0 - 60 µg/ml TCE standard curve is shown in figure 7 yields a linear equation of $y = 33020x + 83188$ with an R-squared value of 0.996.

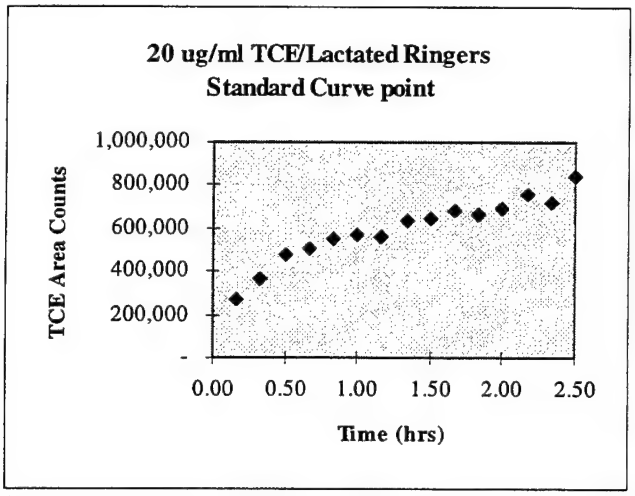


Figure 4: The 20 µg/ml trichloroethylene (TCE)/Lactated Ringers data used for standard curve. Average steady-state area counts are 704,611.

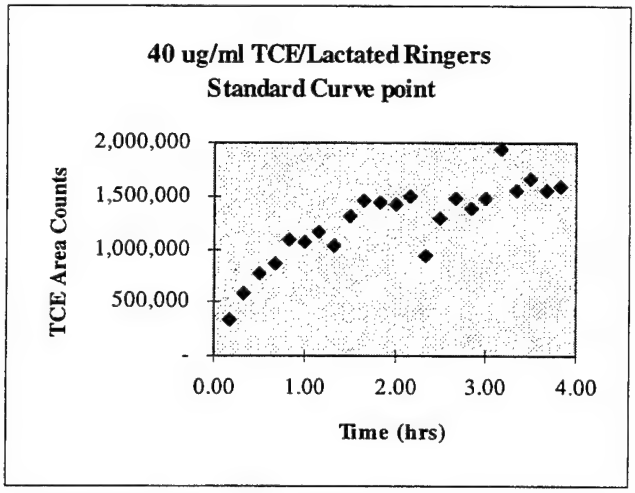


Figure 5: The 40 µg/ml trichloroethylene (TCE)/Lactated Ringers data used for standard curve. Average steady-state area counts are 1,477,439.

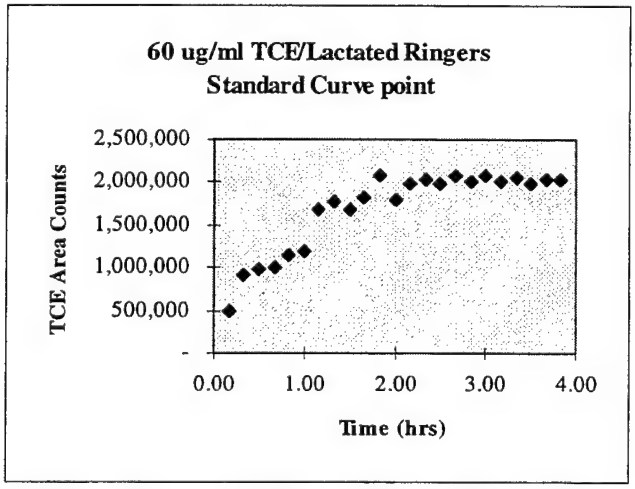


Figure 6: The 60 µg/ml trichloroethylene (TCE)/Lactated Ringers data used for standard curve. Average steady-state area counts are 2,028,436.

TCE Concentration (ug/ml)	Average steady state TCE area counts from probes
0.00	84,695
20.00	704,611
40.00	1,477,439
60.00	2,028,436

Table 1: Summary table of trichloroethylene concentration in microgram per milliliter versus the average steady state trichloroethylene area counts. These results were used for 0 - 60 µg/ml Trichloroethylene/Lactated Ringers standard curve shown in figure 7.

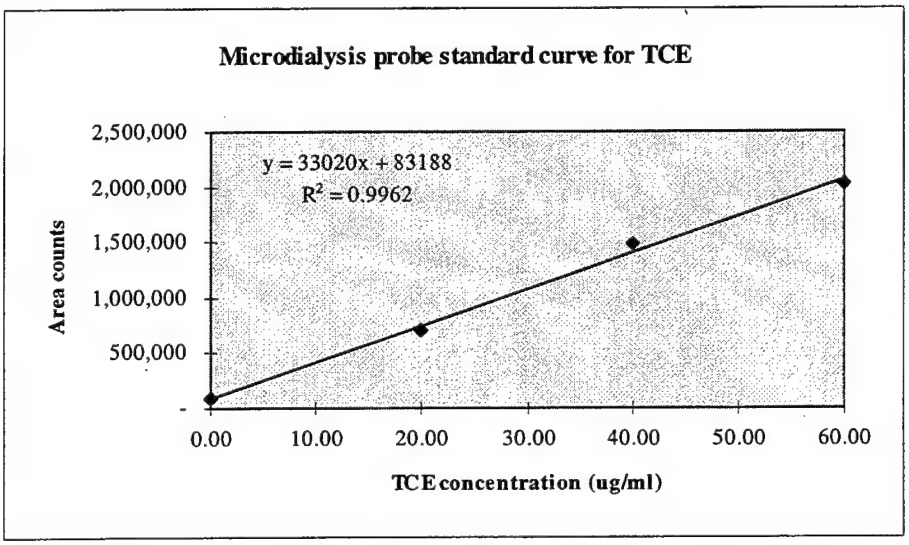


Figure 7: Trichloroethylene/Lactated Ringers standard curve ranging from 0 - 60 µg/ml.

The *in vitro* sampling of TCE from an excised kidney indicated that recovery of TCE from a microdialysis probe was possible. However, the relative recovery was estimated to be 26.8% for both the 20 µg/ml and 40 µg/ml Trichloroethylene concentrations. Flow rates ranging from 2.5 µl/min to 5.0 µl/min were examined, however this did not notably effect the relative recovery. Therefore the flow rate of 5.0 µl/min was chosen, as the minimum sample volume (50 µl) was achieved in a more timely fashion. Nonetheless, as a standard curve would show the same relative recovery of TCE as the *in vivo* samples, this correction factor was not used for the calculation of the *in vivo* TCE concentrations.

The *in vivo* results from an eight hour, 2500 ppm Trichloroethylene nose only exposure are shown in figure 8. These results are typical of those from this study. In this example, the blood concentration reached steady state at 17.214 ± 1.983 µg/ml, while the kidney concentration reached steady-state at 17.476 ± 1.086 µg/ml. Therefore, the kidney/blood partition coefficient for this example was 1.02.

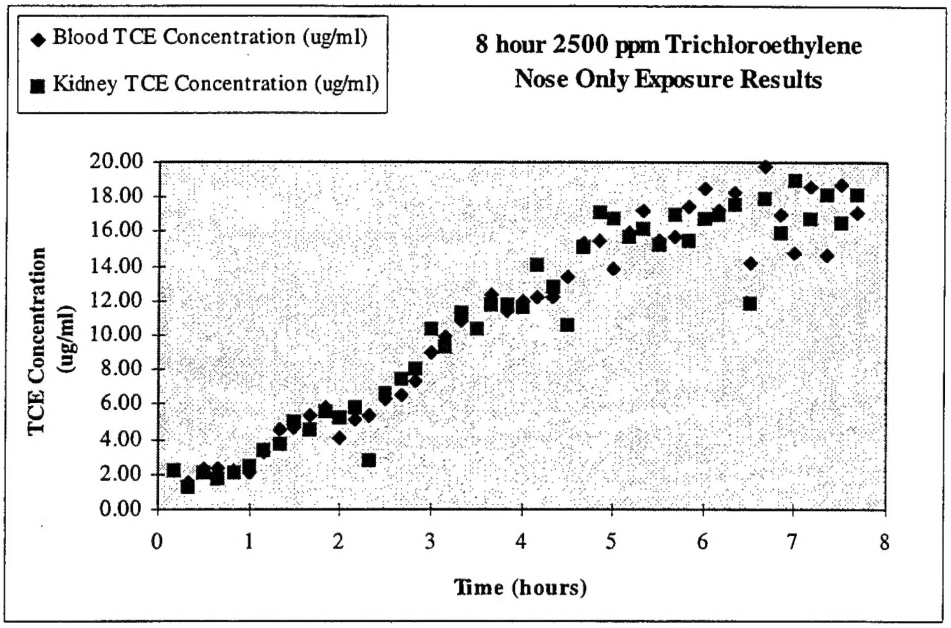


Figure 8: Sample of *in vivo* microdialysis probe results.
The 2500 ppm Trichloroethylene nose-only exposure lasted for eight hours.

A summary of the fat/blood, kidney/blood and liver/blood partition coefficients as determined by *in vivo* use of the microdialysis probes is shown in table 2. *In vivo* exposure concentrations ranged from 2500 ppm to 7500 ppm Trichloroethylene, while the length of exposure varied from 2.5 hours to 8.0 hours. The duration of exposure was usually determined by the length of time that the animal remained alive and properly anesthetized. As shown in figure 8, steady-state is reached after 6 hours of anesthetized nose-only exposure of TCE. Due to the anesthetic itself, and the anesthetic effect of TCE throughout the exposure, it was difficult to refrain from over dosing the animal with either Sodium Pentobarbital or TCE until steady state is reached. TCE concentrations of 5000 ppm and 7500 ppm showed a high incidence of over dose.

In vivo TCE exposure concentration (ppm)	Length of exposure (hrs)	Fat/Blood partition coefficient	Kidney/Blood partition coefficient	Liver/Blood partition coefficient
2500	2.5	n/a	0.98	n/a
2500	4.0	n/a	0.71	n/a
2500	8.0	n/a	1.02	n/a
5000	3.5	n/a	1.67	n/a
5000	2.6	1.59	1.75	n/a
5000	5.8	n/a	**	**
7500	3.0	n/a	**	**
Average	4.2	1.59	1.23	~~
Standard Deviation	2.0	~~	0.5	~~
n=	7	1	5	0

Key to Table 2:

Symbol	Meaning
n/a	Tissue was not sampled.
**	Rat died prior to reaching steady-state conditions.
~~	Information can not be calculated.

Table 2: Summary table of tissue/blood partition coefficients resulting from *in vivo* microdialysis probe use.

As determined by the in vivo microdialysis probe method of partition coefficient determination, the fat/blood partition coefficient was 1.59, and the kidney/blood partition coefficient was 1.23 ± 0.5, table 2. Both studies that sampled the liver TCE concentrations resulted in premature death of the rat. Although there is no numerical information for the liver/blood partition coefficient available, the results from the microdialysis probes that sampled the liver and blood prior to death were similar in appearance to figure 8, indicating that the resulting partition coefficient would have been near one.

DISCUSSION

Microdialysis probes were introduced to pharmacokinetics so that a new method of determining partition coefficients may be possible in an *in vivo* setting. Prior to this report, partition coefficients had been determined for trichloroethylene by use of the vial equilibration technique described earlier [1,8]. According to these sources, the fat/blood partition coefficient was determined to be 25.30, the liver/blood partition coefficient to be 1.20 and rapidly perfused/blood partition coefficient to be 1.20. While the microdialysis probe technique kidney/blood partition coefficient of 1.23 ± 0.5 is close to the vial equilibration technique rapidly perfused/blood partition coefficient of 1.2, it is obvious that the microdialysis probe technique fat/blood partition coefficient of 1.59 does not agree with the vial equilibration technique fat/blood partition coefficient of 25.30.

The explanation for the disparity of these numbers is not known at this time. However, as the sampling of the microdialysis probe is limited to the extracellular space of the tissue and blood, it is possible that TCE is bound in this state but when the tissue or blood is separated from the living system and heated, as in the vial equilibration technique, it is released. Alternatively however, the reason for the differences in partition

coefficients between methods may be a result of an undetermined flaw that further investigation may resolve. This flaw could originate anywhere from sample analysis to a flaw in the general theory.

Apparent problems which may influence the partition coefficients include the occurrence of death resulting from exposing anesthetized rats to trichloroethylene. In order to eliminate the possibility of over dosing the animal, other possibilities, such as exposing non-anesthetized animals which have been previously implanted with microdialysis probes, should be examined. In addition, chemical selection should be considered as a potential uncertainty. Hydrophobic chemicals such as trichloroethylene, generally have a relative recovery of 25-30% [10]. Results from this study confirm this, as the relative recovery was found to be 26.8%. Although this should not have an effect in the partition coefficient as it is a ratio of two concentrations, it should be considered if tissue concentration is examined alone. For investigative purposes however, examining a hydrophilic chemical may have merit. Finally, as the analysis of the microdialysis probe samples is a lengthy process, it too should be closely investigated for improvement possibilities.

CONCLUSION

At this time, the microdialysis probe method for determining partition coefficients *in vivo* is not a viable replacement for the established vial equilibration technique. If upon further investigation the irregularities of the system are resolved, it may become an adequate method for determining partition coefficients *in vivo* to be used collaboratively with the *in vitro* vial equilibration technique.

REFERENCES

1. Gargas ML, Burgess RJ, Voisard DE, Cason GH and Andersen ME. Partition Coefficients of Low-Molecular-Weight Volatile Chemicals in Various Liquids and Tissues. Toxicology and Applied Pharmacology. 98:87 - 99, 1989.

2. Sato A, and Nakajima T. Partition coefficients of some aromatic hydrocarbons and ketones in water, blood, and oil. Brit. J. Ind. Med. 36:231 - 234, 1979.

3. Lunte CE, Scott DO, and Kissinger PT. Sampling Living Systems Using Microdialysis Probes. Anal. Chem. 63:773-780, 1991.

4. Hernandez L, Stanley BG, and Hoebel BG. A small, removable microdialysis probe. Life Sciences. 39:2629-2637, 1986.

5. Wages SA, Church WH, and Justice Jr. JB. Sampling Considerations for On-Line Microbore Liquid Chromatography of Brain Dialysate. Anal. Chem. 58:1649-1656, 1986.

6. Bioanalytical Systems Inc. 1992 Catalog. pp 111.

7. Bioanalytical Systems Inc. 1992 Catalog. pp 132.

8. Fisher JW, Gargas ML, Allen BC, and Andersen ME. Physiologically Based Pharmacokinetic Modeling with Trichloroethylene and Its Metabolite, Trichloroacetic Acid, in the Rat and Mouse. Toxicology and Applied Pharmacology. 109:183-195, 1991.

9. Salins SA. Method of Cannulation of Jugular Veins in Rats. Toxic Hazards Research Unit Standard Operating Procedure. SOP NO. 4300-429, 1991.

10. Kissinger, C. 1994. Technical Representative of Bioanalytical Systems, West Lafayette, IN. Personal Communication.